

MICROSTIMULATION OF LUMBOSACRAL SPINAL CORD- MAPPING

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**1st Progress Report
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**Prepared by
James R. Roppolo, PhD.**

University of Pittsburgh
School of Medicine
Pittsburgh, PA 15261

I. Introduction

During this quarter we continued studies begun previously which used several (2-4) fine tipped microelectrodes to stimulate the lumbosacral spinal cord to produce extension and flexion of the hindlimb about the knee joint. These studies will be the subject of future progress reports. The aims of the present progress report are to present data on new experiments begun during this quarter. These studies examined the use of single and multiple electrodes to map colon responses to microstimulation of the sacral spinal cord. The results from these experiments indicate that microstimulation of the S₂ segment of the sacral spinal cord produces increases in distal colon pressure when the tip of the microelectrode is near or in the sacral parasympathetic nucleus, but is almost without effect when sites deep within the ventral horn are stimulated.

During this quarter new tracing experiments were also begun to examine the location and distribution in the spinal cord of neurons and interneurons involved in the control of colon smooth muscle activity. These studies indicate that neurons which project to the colon are located in the lumbar and sacral segments of the spinal cord. Their location within the spinal cord is consistent with what is known about the parasympathetic and sympathetic innervation of the distal colon. The details of these studies are described below.

II. Changes in Distal Colon Pressure Produced by S₂ Spinal Cord Microstimulation

These studies were designed to determine sites in the sacral spinal cord which produce changes in distal colon activity. Since the sacral spinal cord provides the major excitatory input to the colon via its parasympathetic innervation, enhanced motility and increases in intraluminal pressure were predicted.

Methods

The methods used in these studies are similar to those described in previous progress reports

and are briefly described below. There are however specific methods used to record colon pressure that are new to these microstimulation studies which will be described in detail.

Adult male cats were anesthetized with α -chlorolose (60-70mg/kg i.v.) and supplemented throughout the experiment as necessary. (α -Chlorolose anesthesia, rather than pentobarbital, was used in this series of experiments in order to better maintain autonomic nervous system reflexes.) A dorsal laminectomy exposed the lumbosacral spinal cord (L₄ to S₃) and roots. A latex balloon (7cm in length and 1.5 cm in diameter) attached to the end of a catheter was placed in the lumen of the distal colon via a small incision in the proximal colon wall. The catheter was secured to the colon and abdominal walls with sutures. The balloon catheter was connected to a pressure transducer for recording intraluminal pressure from the colon. A syringe attached to a side arm of the catheter was used to fill or empty the balloon as needed. During mapping studies the balloon was inflated to a pressure of 8-10cm H₂O and maintained throughout the experiment. The intraluminal pressure was displayed on a chart recorder, stored on tape and also digitized and stored in a computer for later analysis (Figure 1).

Fine tipped (≈ 400 sq. μ m exposed area) activated iridium electrodes were used to stimulate the sacral spinal cord at various depths using increments of 200 μ . Each electrode tract was separated from an adjacent tract by a minimum of 300 μ . At the end of each experiment the spinal cord is fixed, sectioned, stained and the electrode tracts identified. The physiologic response is then matched with the anatomical site in the spinal cord.

Off-line analysis of colon intraluminal pressure changes consists of determining peak pressure, area under the stimulus response curve, response duration (response often outlasts stimulus) and latency to initial pressure changes.

Results

At the beginning of an experiment each sacral ventral root is stimulated with a hook electrode

to determine the sacral segment which provides the major excitatory outflow to the distal colon. Typically the S_2 ventral root produces the largest pressure rise in the distal colon while S_1 and S_3 produces either smaller pressure rise or no response. Figures 2 and 3 shows the pressure changes to a 30 sec. ventral root stimulation at four intensities of stimulation. Figure 2 illustrates S_1 ventral root stimulation while Figure 3 shows the response to S_2 . Notice that at the same stimulus intensity the S_2 response is more than two times as large as the S_1 response. In this particular experiment the S_3 root gave no response. The latency to the beginning of the pressure change was usually short, typically less than 3 seconds. Following termination of the stimulus the pressure slowly returned to baseline over several seconds and varied somewhat with intensity of stimulation. Figure 4 shows the response to S_1 and S_2 ventral root stimulation at various intensities and for S_2 at various frequencies of stimulation. In Figure 4 three types of data are shown (1) peak pressure response for a 30 second stimulation of the ventral root, (2) area under the pressure response curve for a 30 second stimulus and (3) duration of the pressure change for a 30 second stimulus. Notice that pressure response and duration increases with increasing stimulus intensity, while stimulus frequency has a smaller effect on the response. The frequency which produces the largest pressure change for a given intensity (10 volts) is between 10-20 Hz.

These preliminary studies using ventral root stimulation provide the starting point for examining the colon responses to microstimulation of the spinal cord. Since S_2 ventral root stimulation produced the largest colon pressure response, the S_2 spinal segment should produce significant increases in distal colon pressure to cord microstimulation. In addition, since ventral root stimulation seems to produce a good response at 20 Hz stimulus frequency; this frequency was used as a starting point for the cord microstimulation experiments.

Figure 5 shows the changes in distal colon pressure to S_2 spinal cord microstimulation at six depths from the S_2 cord surface. The response was near maximal at 1.4 mm from the S_2 cord surface.

This area corresponded to the area of the sacral parasympathetic nucleus. At 2.2 mm from the surface there was no response to a 30 sec. stimulus ($100\ \mu\text{A}$, 0.2 msec, 20 Hz). Notice that responses in general were considerably smaller than ventral root activation. Also the colon response to cord stimulation had a sharp rise in pressure followed by a partial recovery. Complete recovery to baseline did not occur until stimulus was terminated. The latency to first pressure increase occurred typically less than four seconds, similar to that seen with ventral root stimulation. Figure 6 shows the colon pressure responses to stimulation at different depths along the four electrode tracts (ABCD). The electrodes were 1.5 mm apart with A the most rostral and D the most caudal. Since this data was obtained from a parallel array of four fixed electrodes the most rostral electrode (A) was slightly medial in the cord while caudal electrode (D) was slightly more lateral. This occurs because the cord is becoming smaller as one moves from rostral to caudal in S_2 . Histologically all electrodes were close to and touched part of sacral parasympathetic nucleus (SPN). The dramatic difference in responses may depend on the rostrocaudal level in the SPN since all four electrodes are in or close to the SPN in medial lateral direction. Notice however that the major response is seen at 0.8 to 1.8 mm which is consistent with the location of the cell bodies of the preganglionic neurons of the SPN. It might be expected that deeper sites in the spinal cord may activate fibers leaving the ventral horn and produce an increase colon pressure. This was seen with bladder axons and motor axons of the hindlimb but not with colon axons. The preganglionic axons to the colon are smaller in diameter than those of the bladder and therefore may have a higher threshold for activation.

These studies will continue in the next quarter and will record bladder and colon activity simultaneously.

III. Pseudorabies Virus Tracing of Neurons and Interneurons In the Lumbosacral Spinal Cord Which Control Colon Activity

The purpose of these tracing studies is to determine the location of spinal cord neurons and interneurons which control the activity of the colon. Of special interest are the excitatory neurons and interneurons in the sacral spinal cord which project to the colon. The location of these neurons may provide important sites for microstimulation.

The methods used for these types of studies have been described in previous progress reports and are briefly described here for colon injection.

Male cats were anesthetized with halothane. The colon is visualized via a midline incision of the abdominal wall. The entire length of the colon, except for the distal 4 cm and the proximal 6-8 cm are injected with multiple (40-50) small ($5\ \mu\text{l}$) injections of pseudorabies virus (PRV). The distal end of the colon is not injected in order to avoid the striated muscle of the external anal sphincter (EAS), which will be the subject of a separate study. The proximal end of the colon is avoided to prevent spread to small intestine. In addition the proximal colon may receive dual input from the sacral spinal cord and brainstem via the vagus. This again will be the subject to a separate study.

The injected PRV is allowed to transport to the spinal cord via its parasympathetic and sympathetic innervation. The transport time is varied from 72 hours to 120 hours. Since PRV can cross synapses, the pathway within the central nervous system which is involved with colon activity is labeled. The labeled neurons include first order as well as interneurons in the pathway projecting to the colon.

The PRV labeled neurons are visualized with antibodies to the virus tagged with fluorescent probes (Cy3, FITC, etc.) and can be observed with a microscope.

Figure 7 shows the distribution of PRV labeled neurons and interneurons in the lumbosacral spinal cord. Both parasympathetic (sacral spinal cord) and sympathetic (lumbar spinal cord) preganglionic neurons are labeled. The parasympathetic preganglionic are primarily in the SPN of

the S₂ and S₃ segments of the spinal cord. The neurons in the SPN are probably a mixture of first order preganglionic neurons and interneurons. The neurons near the central canal are most likely interneurons. Their role in regulating colon activity whether excitatory or inhibitory is unknown. The neurons in the intermediolateral cell column (IML) of the lumbar spinal cord are probably a mixture of sympathetic preganglionic neurons and interneurons, while those around the central canal may represent interneurons.

The preganglionic neurons in the S₂ segment are probably important sites which provide excitatory input to the colon. These neurons have been and will continue to be targets for our microstimulation experiments. The fact that the location of the preganglionic neurons to the colon seem to be more medial and dorsal in the SPN and not as dense as those to the bladder, may account for the smaller colon responses seen with cord stimulation.

These tracing studies will continue in the next quarter.

- Figure 1.** Schematic diagram of the experimental setup, showing the colon with balloon catheter in the distal colon. Pressure is recorded from saline filled balloon via a transducer (trans). Pressure recordings are displayed on chart recorder and recorded on tape and on computer.
- Figure 2.** Chart recorder tracings showing changes in colon pressure to stimulation of the S₁ ventral roots at four different stimulus intensities (0.1, 0.5, 2.0, and 10 V). Stimulus duration was 30 seconds in duration and is marked on the graph by arrows. Stimulus parameters are marked in each panel. 10 V produced the maximal response.
- Figure 3.** Same as Figure 2 except for S₂ ventral root.
- Figure 4.** Graphs showing the changes in colon responses for increasing intensities of stimulation for S₁ and S₂ ventral roots (top and middle panel respectively) and for increasing frequencies of stimulation of S₂ ventral roots (bottom panel). Three types of response characteristics are shown: (1) peak pressure, (2) area under the pressure response curve, (3) duration of pressure change. Stimulus parameters are: 20 Hz, 0.05 msec pulse for 30 seconds, at 0.1 to 25 volts (top and middle panel); 0.05 msec pulse, 10 volts, 5-40 Hz, bottom panel.
- Figure 5.** Chart record tracing showing changes in colon pressure to stimulation (arrows) of the S₂ spinal cord at six depths (1.2 to 2.2 mm) from the cord surface. Stimulus parameters are: 0.2 msec pulses, 20 Hz, 100 μ A, 30 seconds on (marked on each graph by arrows).
- Figure 6.** Graphs showing the changes in colon responses at various depths (0.4 to 3.0 mm) from S₂ spinal cord surface along four electrode tracts (1A, 1B, 1C, and 1D) 1.5 mm apart in the rostrocaudal direction. As in Figure 4, three colon responses are shown, area, peak, and duration. Stimulus parameters are: 0.2 msec pulses, 20 Hz, 100 μ A, 30 seconds on, 120 seconds off.
- Figure 7.** Camera lucida drawing at three levels each of the sacral and lumbar spinal cords, showing the location and distribution of pseudorabies virus (PRV) labeled neurons following injection into the colon. Notice the labeled neurons in the sacral parasympathetic nucleus of S₂r and S₃r and around the central canal of S₁, S₂, and S₃. Also notice the PRV labeled neurons in the intermediolateral (IML) cell column of L₂, L₃, and L₄. The cells in the IML are at least in part sympathetic preganglionic neurons projecting to the colon.

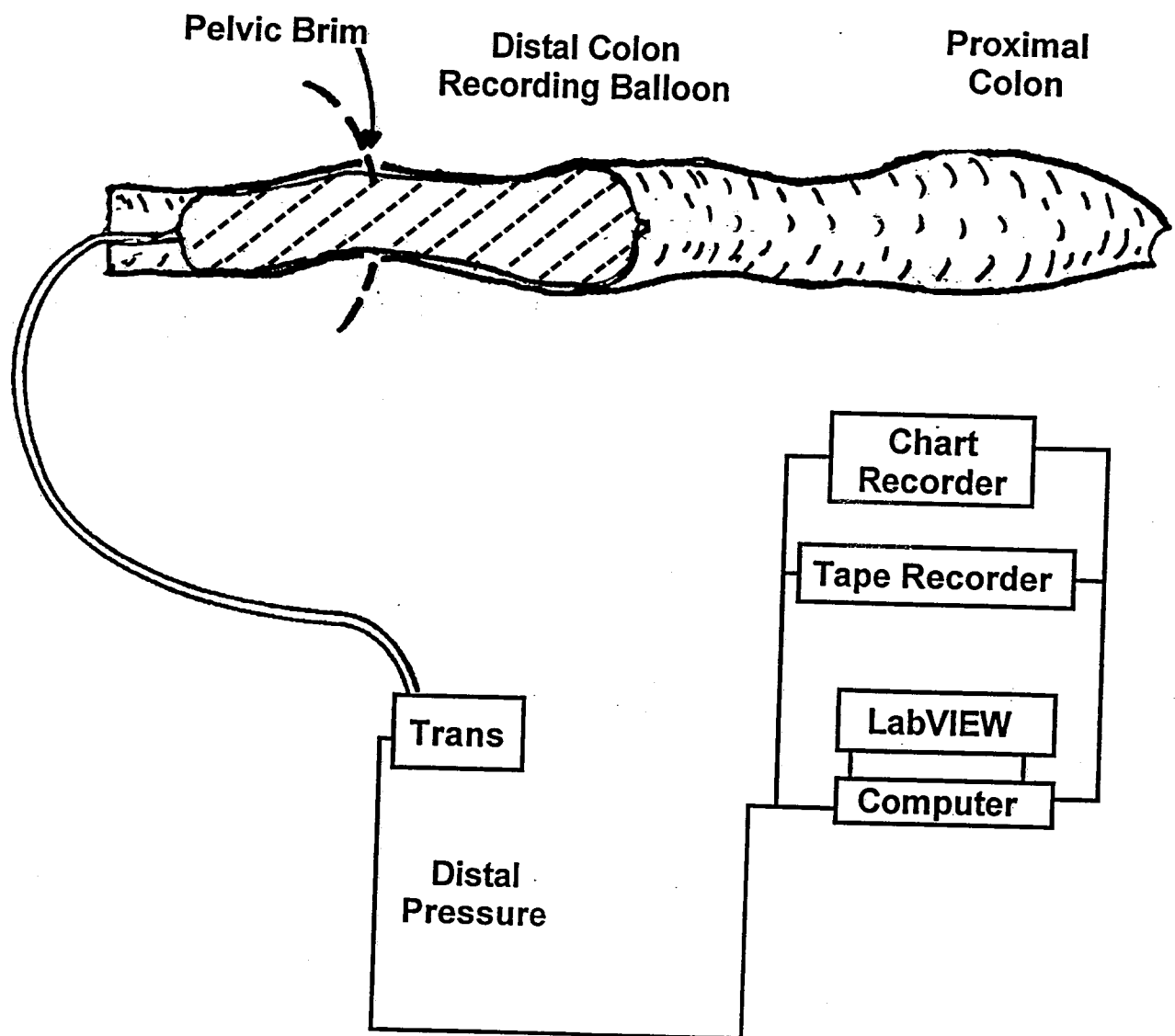


Figure 1

S₁ Ventral Root

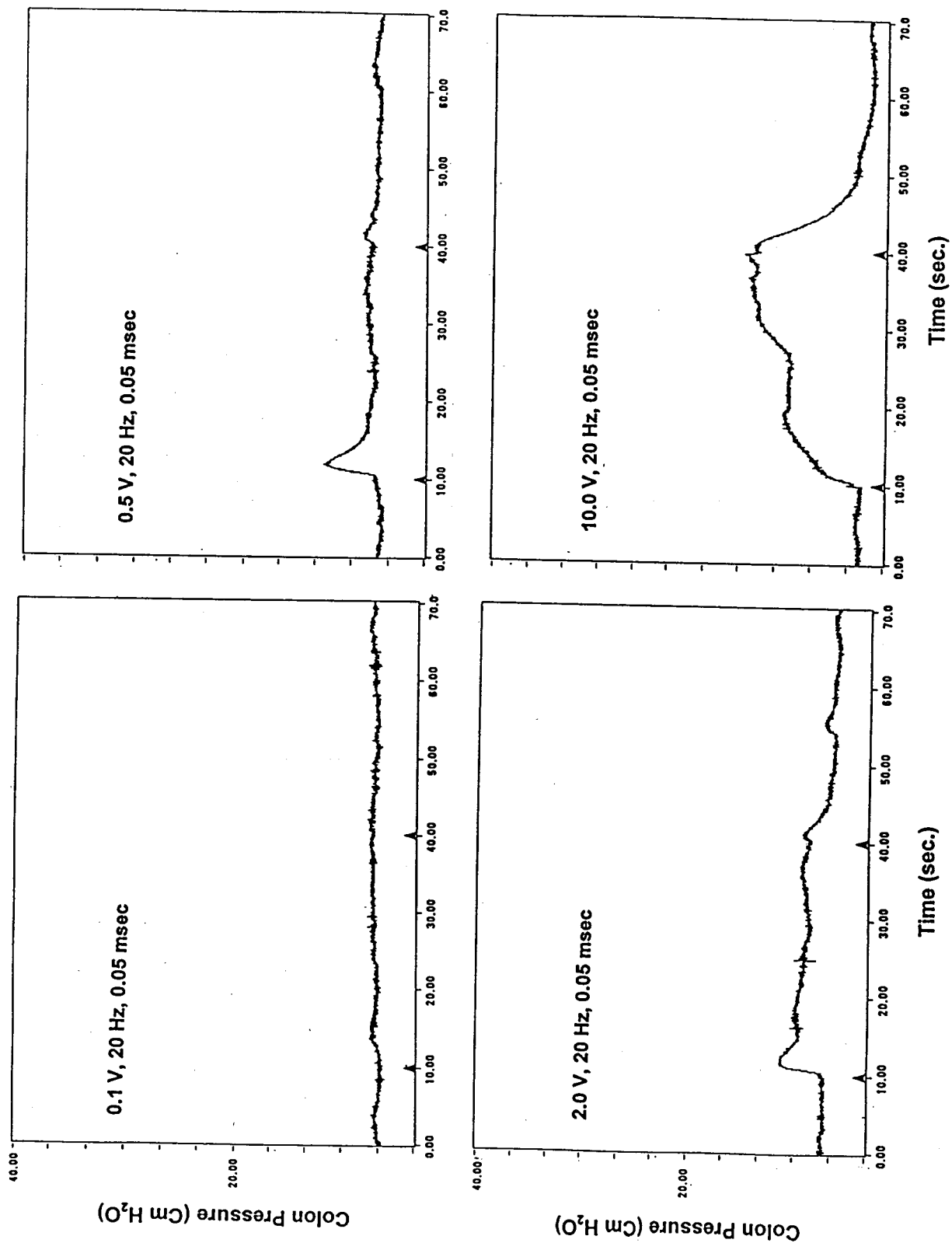


Figure 2

S₂ Ventral Root

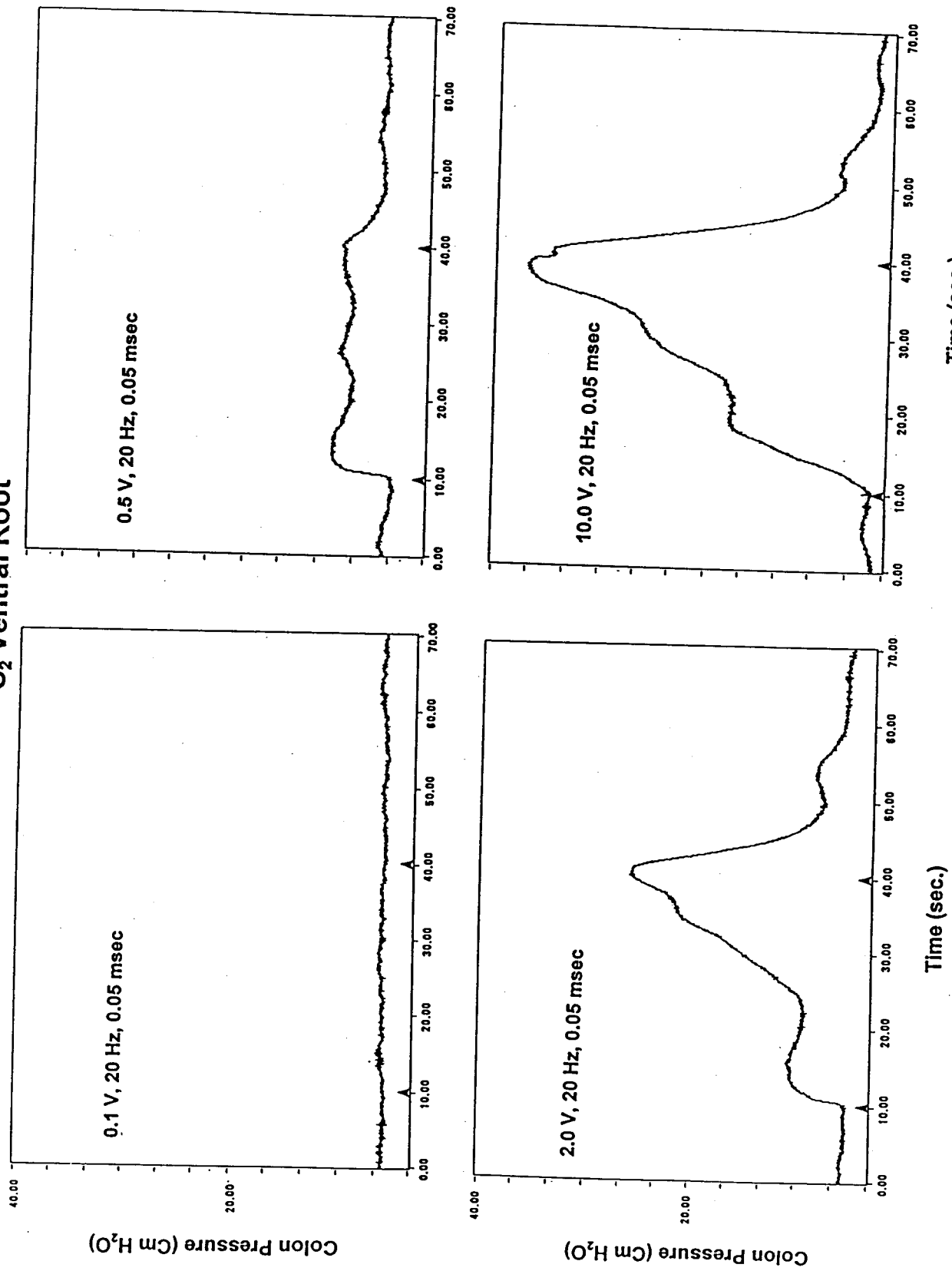
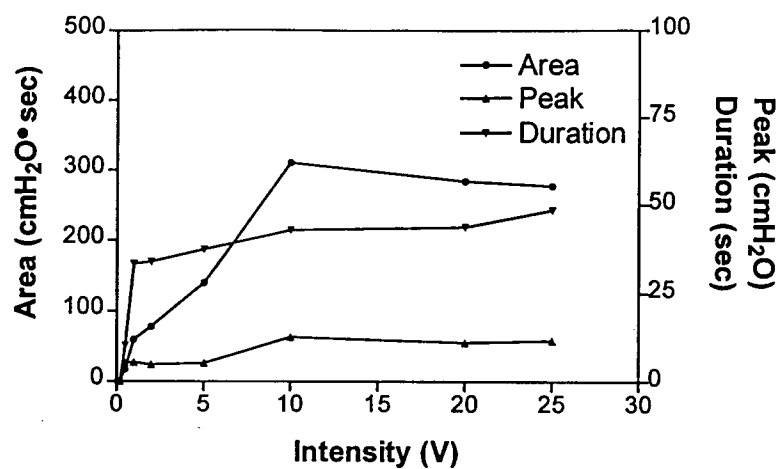
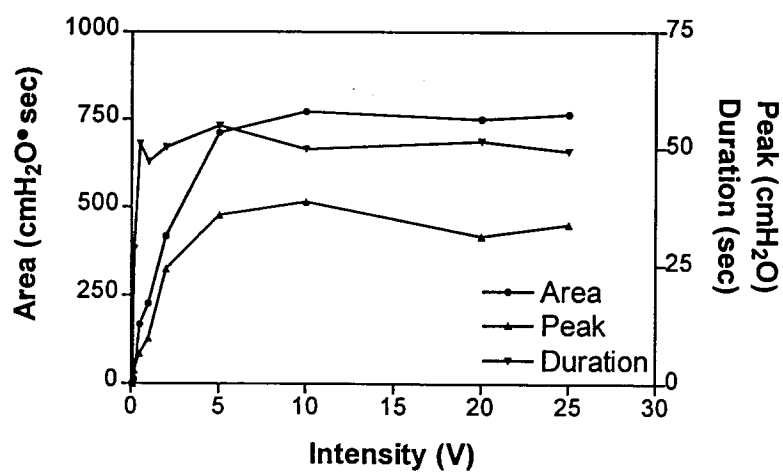


Figure 3

S1 Left VRT



S2 Left VRT



S2 Left VRT

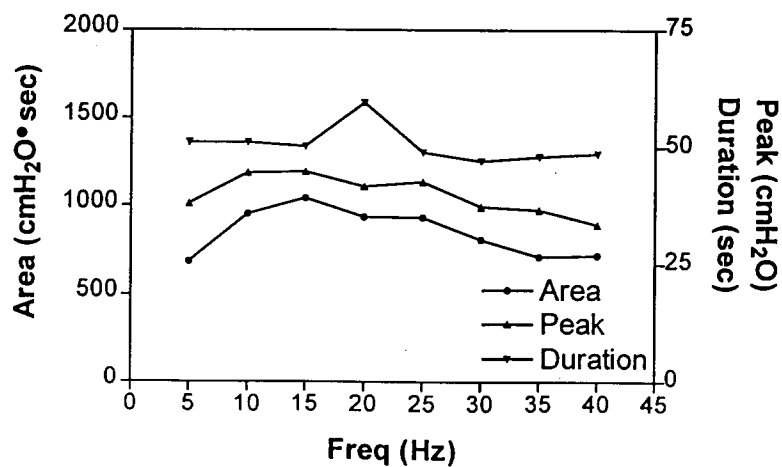


Figure 4

S₂ Spinal Cord Stimulation

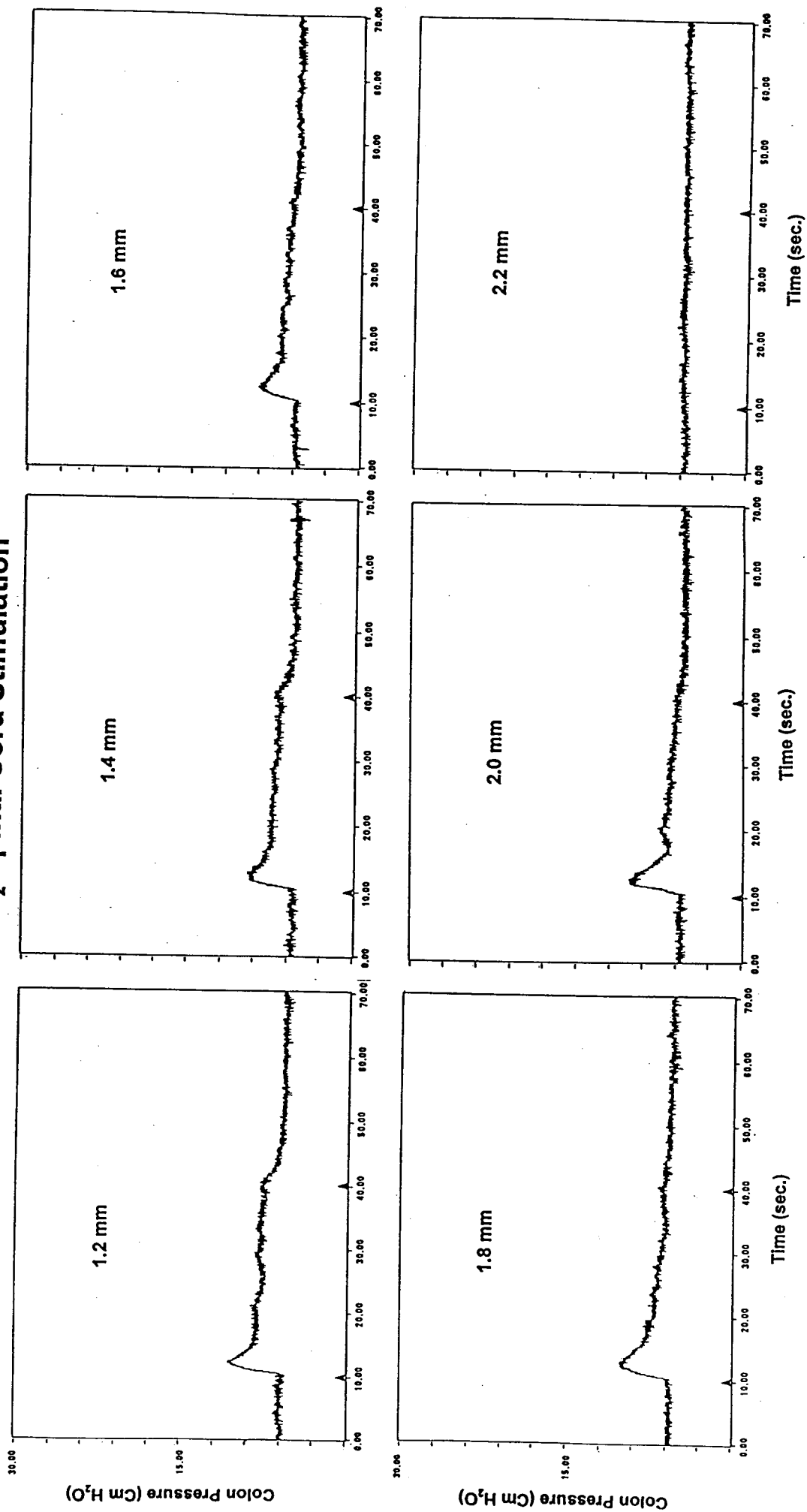


Figure 5

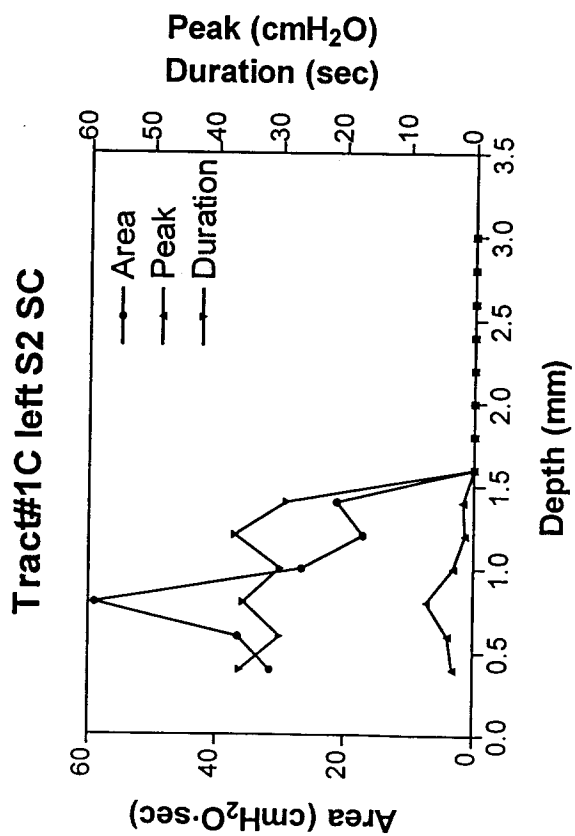
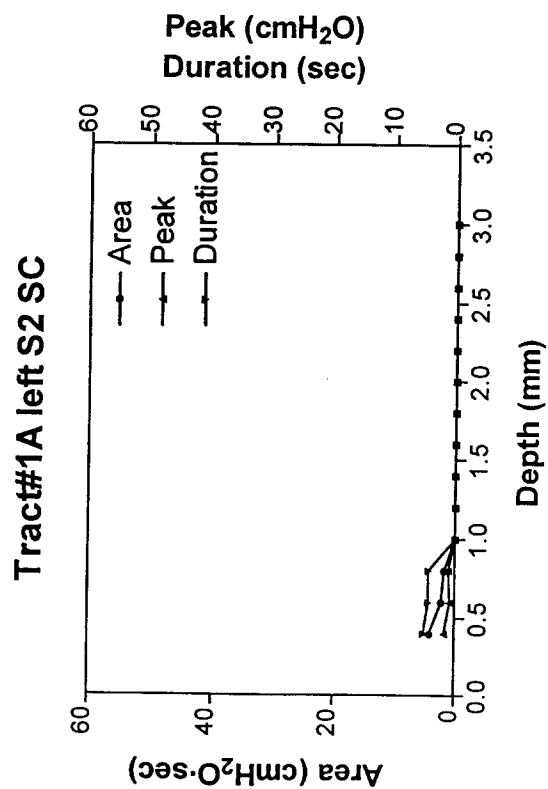
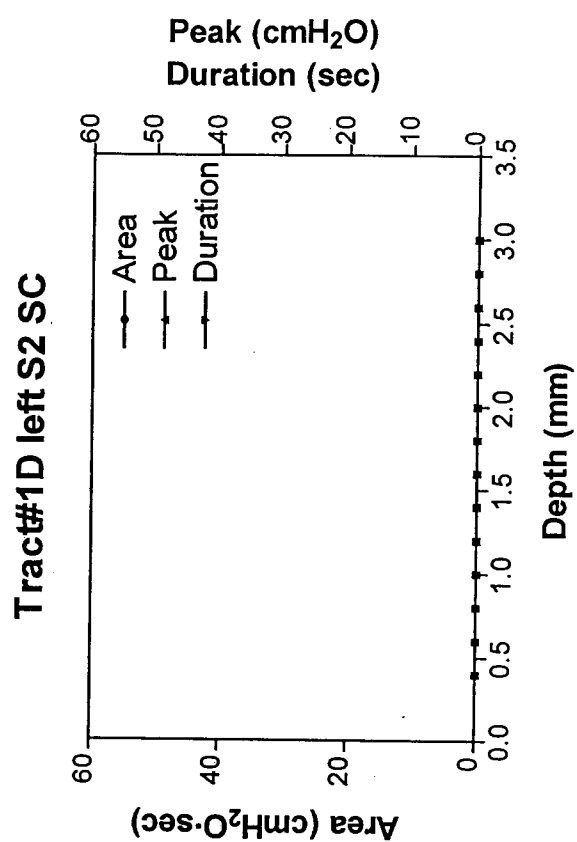
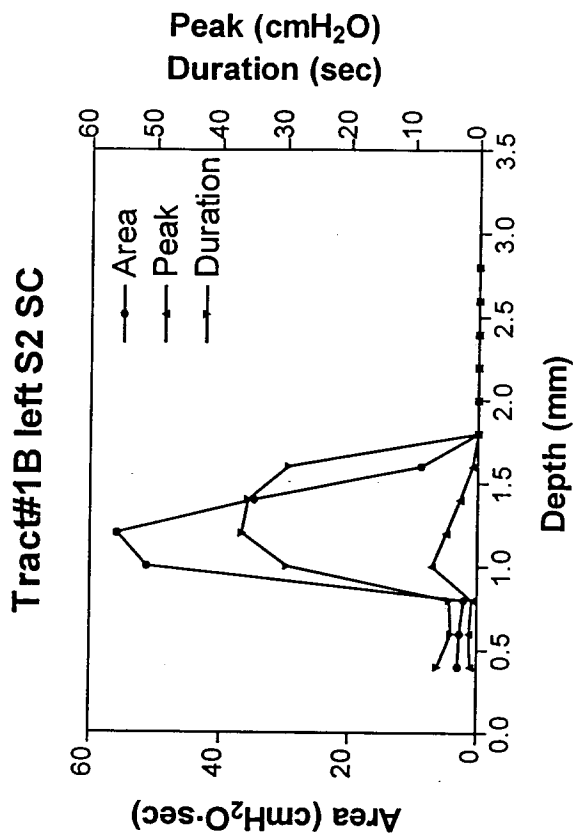


Figure 6

PRV into Colon

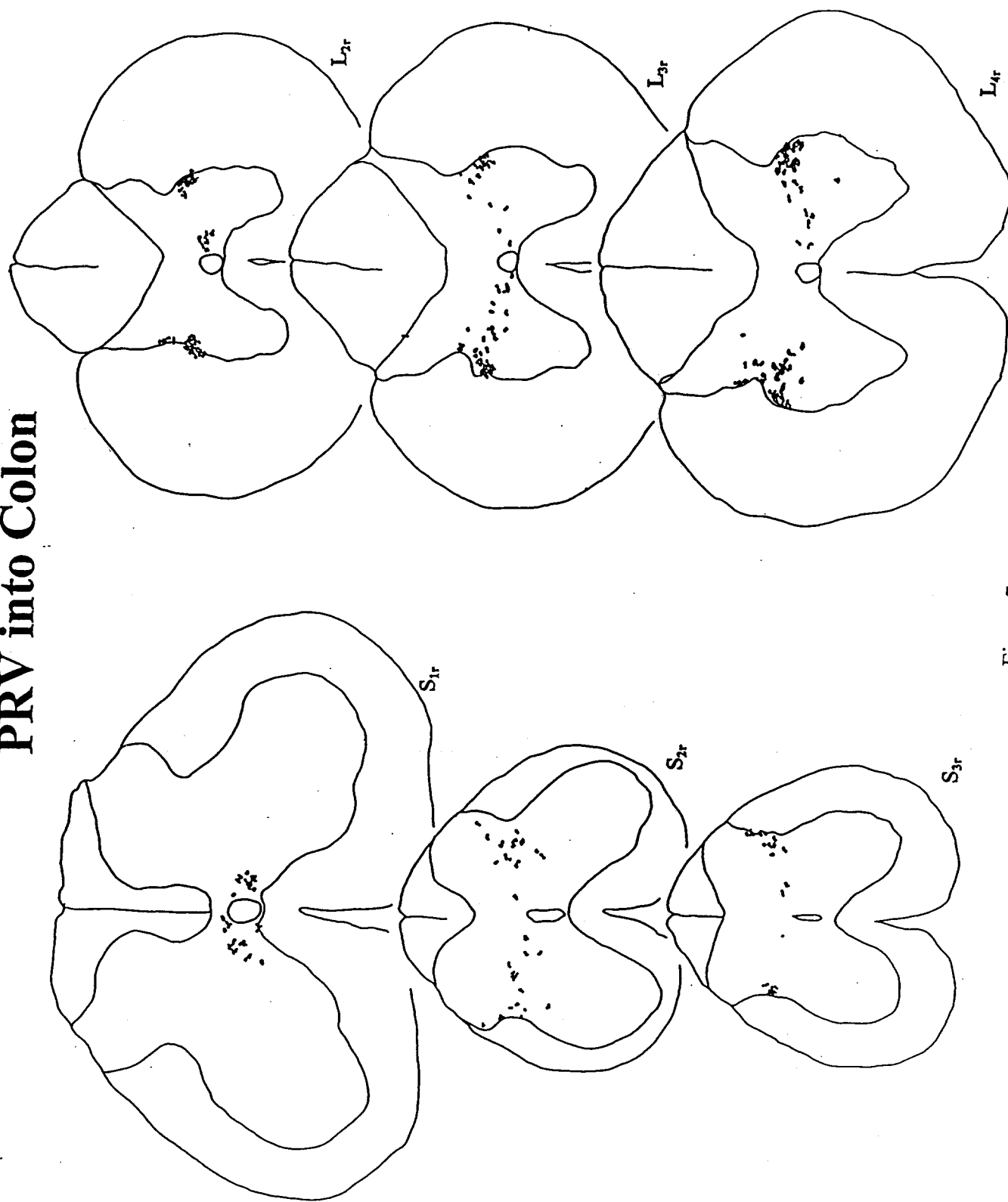


Figure 7